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ANALYSIS OF MONITORING STUDIES ON AFRICAN SWINE FEVER VIRUS DNA DETECTION CARRIED OUT IN 2017

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SUMMARY

The paper describes the results of testing biomaterial from domestic pigs and wild boars by real-time PCR used for African swine fever virus genome detection, carried out in the FGBI "Federal Centre for Animal Health" (Vladimir). In 2017 8,500 samples from 44 subjects of the Russian Federation were tested within the framework of the state laboratory monitoring. African swine fever virus genome was detected in 504 samples. In 2017 ASF outbreaks were registered in the Urals and Siberian Federal Districts of the RF for the first time. The conducted research and persistent ASF infection in the territory of the RF have demonstrated the need for further surveillance in the populations of susceptible animals. Development, organization and implementation of the program for ASF spread surveillance in wild fauna remains a high priority. It is necessary to create and implement sampling schedules with uniform sampling of biomaterial and submission of the collected samples to the research laboratories for timely ASF outbreak containment at the regional level.

Key words: African swine fever, monitoring studies, genome, real-time polymerase chain reaction.

INTRODUCTION

The RF food safety is one of the principle aspects of national safety provision in the medium term [4]. Within this aspect the following set of measures aimed at animal husbandry developments is envisaged. Among them – improvement of epidemic situation of highly dangerous animal diseases including African swine fever (ASF) [3].

Prevention of the infection spread and improvement of health situation in the country directly depends on the effectiveness of the surveillance system in place [1].

The surveillance is targeted at:

1) finding evidence of the disease/infection absence/presence;

2) determining the trend of the disease development in susceptible populations;

3) previous detection of foreign and emerging diseases [12].

Since the beginning of the ASF epidemics in 2007–2008 1,274 outbreaks were reported in the RF 50% of which were reported in the domestic pig population from small farms (backyard farms, private farms) [9]. Up to 2011 the infection outbreaks were mostly reported in the Southern and North-Caucasian Federal Districts where the first ASF endemic zone "South" was established. In 2012–2013 the disease was reported both in the Southern and in the Central Federal District, where as a result the second ASF endemic zone "North" was established.

Subsequently, the infection spread trend covering new areas continued [9]. Thus, since 2015 the next stage of ASF epidemics began – the third wave of the epidemic process – which was accompanied by considerable increase in the number of disease outbreaks and spread into new Subjects. As a result within 10 years ASF extended to 43 Subjects of the European Part of the RF [8].

Today the risk of further spread of the infection is still high and it is associated with the following factors: 1) inter-farm supplies of live animals (pigs) and pig products from ASF infected zones to ASF free territories if there are delays in the chain "diagnosis-quarantine"; 2) partial or complete

absence of biosecurity measures on small farms (backyard farms or private farms) due to lack of financial resources and misunderstanding as well as reluctance to perform surveillance; 3) absence of preventive programs in several RF Subjects; 4) movement of live animals and pig products without veterinary documents; 5) diffuse infection spread within the infected subject and out of it due to wild boar migration (intra-regional factor) [8].

An important aspect of surveillance measures are rapid tests of biological material samples (pathological material and blood serum) for ASFV or its genetic material as well antibodies against the agent performed by the laboratories (testing centres) of the RF National Veterinary Service or laboratories (testing centres) accredited in the national accreditation system [2].

In the present time the laboratory tests demonstrate that PCR remains the "first choice" method for ASF diagnosis [1].

The work was aimed at analysis of the monitoring tests for ASFV DNA detection performed in the FGBI "ARRIAH" reference laboratory for ASF in 2017.

MATERIALS AND METHODS

The object of the molecular and genetic tests was genetic material from domestic pigs and wild boars and samples from pig products delivered to the FGBI "ARRIAH" from different RF Subjects in 2017.

Total DNA extraction was performed by nuclear absorption using commercial reagent kit for DNA extraction from clinical material "AmpliPrime DNA-Sorb-B" (OOO "NextBio", Moscow).

RT-PCR was performed using "ASF" test kit for ASFV detection (Rospotrebnadzor FBUN, Moscow) according to the attached manufacturer's instructions [5, 6].

RT-PCr was performed using Rotor-Gene 6000 amplifier (Qiagen, Germany). The results were recorded by analyzing

Table 1
Samples delivered to the FGBI "ARRIAH" Veterinary Laboratory for testing, RF Subjects, 2017

No.	RF Subject	Number of samples		No.	RF Subject	Number of samples	
		Total	Positive			Total	Positive
1	Arkhangelsk Oblast	92	0	23	Novgorod Oblast	199	0
2	Astrakhan Oblast	92	0	24	***Omsk Oblast***	106	**97 (91.51%)**
3	Belgorod Oblast	212	0	25	Orenburg Oblast	100	0
4	Bryansk Oblast	100	0	26	***Oryol oblast***	91	**12 (13.19%)**
5	***Vladimir Oblast***	778	**52 (6.68%)**	27	Penza Oblast	200	0
6	***Volgograd Oblast***	206	**2 (0.97%)**	28	Pskov Oblast	100	0
7	Vologda Oblast	750	0	29	***Rostov Oblast***	84	**4 (4.76%)**
8	Voronezh Oblast	200	0	30	Ryazan Oblast	185	0
9	Zabaykalsky Krai	69	0	31	Samara Oblast	95	0
10	Ivanovo Oblast	472	0	32	Smolensk Oblast	100	0
11	***Irkutsk Oblast***	11	**5 (45.46%)**	33	Stavropol Krai	195	0
12	***Kaliningrad Oblast***	512	**7 (1.37%)**	34	Tver Oblast	100	0
13	Kaluga Oblast	200	0	35	Tula Oblast	100	0
14	Kirov Oblast	223	0	36	***Tyumen Oblast***	8	**8 (100.00%)**
15	Kostroma Oblast	313	0	37	***Chelyabinsk Oblast***	17	**11 (64.71%)**
16	***Krasnodar Krai***	88	**35 (39.77%)**	38	Yaroslavl Oblast	103	0
17	***Kurgan Oblast***	21	**3 (14.29%)**	39	Republic of Bashkortostan	300	0
18	Kursk Oblast	100	0	40	Republic of Dagestan	9	0
19	Leningrad Oblast	60	0	41	***Republic of Crimea***	914	**262 (28.67%)**
20	Lipetsk oblast	200	0	42	Republic of Mordovia	304	0
21	Moscow Oblast	70	0	43	Republic of Udmurtia	200	0
22	***Nizhny Novgorod Oblast***	6	**6 (100.00%)**	44	Republic of Chuvashia	205	0

the fluorescence signal curve for each sample using software.

RESULTS AND DISCUSSION

According to the Rosselkhoznadzor Order No. 1004 as of December 30, 2016 (considering amendments in 2017) "On laboratory tests performed in 2017 in the framework of Rosselkhoznadzor activities to comply with requirements of the WTO-SPS Agreement at Russia's joining the WTO" (http://www.refcenter57.ru/files/1004.pdf) the specialists of the FGBI "ARRIAH" laboratory for ASF carried out tests of biological material from domestic pigs and wild boars as well as pig products.

In 2017 8,500 samples (pathological material, blood and sera from domestic pigs and wild boars, pig products) from 44 RF Subjects (Table 1) were tested. The tests were performed within active monitoring to confirm ASF diagnosis made by the regional veterinary laboratories and interregional veterinary laboratories subordinate to the Rosselkhoznadzor.

Due to the tense epidemic situation most samples were sent for testing from the Republic of Crimea (914 samples) and Vladimir Oblast (778 samples). The least number of samples were delivered from the Republic of Dagestan, Nizhny Novgorod and Tyumen Oblasts (Table 1).

The peak of delivered samples was in September and November 2017 – 1,411 and 1,884 samples respectively, which is primarily associated with the necessity to accomplish planned anti-epidemic activities. The increase in the number of samples in the II–IV quarters of the reference period was also caused by the following factors: 1) natural factor, driving ASF morbidity increase due to biological characteristics of animals (increase of intra- and inter-population contacts during mating period, wild-boar herd regrouping, etc.); 2) anthropogenic factor leading to the growth of outbreaks in spring and summer (active purchase of fattening piglets, peculiarities of pig management in unsecured holdings (backyard farms, private holdings) (Figure 1).

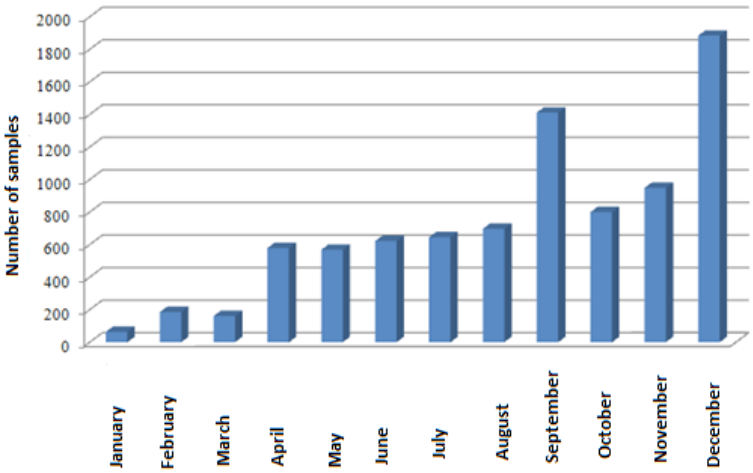

Figure 1. Samples received for sampling, by months, 2017

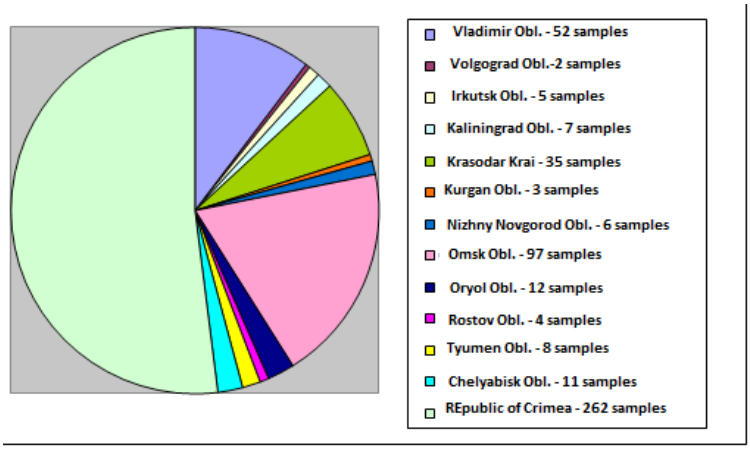

Fig. 2. Samples containing ASFV genome, by Subjects

As a result of the tests performed 506 samples containing ASFV DNA (5.93% of all samples) were detected. The specified samples were delivered from the following RF Subjects: Vladimir, Volgograd, Irkutsk, Kaliningrad, Kurgan, Nizhny Novgorod, Omsk, Oryol, Rostov, Tyumen, Chelyabinsk Oblasts, Krasnodar Krai and the Republic of Crimea (Fig. 2). Considering the data on the epizootic situation in the previous years as well as the results of this research it can be concluded, that continuous ASF infection persists in three subjects: in Krasnodar Krai the outbreaks have been reported since 2008, in Rostov Oblast – since 2009 and in Volgograd Oblast – since 2010. In Oryol Oblast the infection outbreaks have been annually reported since 2014. The first ASF report from the Nizhny Novgorod Oblast was received in 2011 (two outbreaks in domestic pigs were reported). Next 4 years the Subject remained ASF free, but in 2016 the secondary introduction of the infection agent occurred. In Vladimir Oblast the disease outbreaks were reported in 2013, 2015 and 2016 [11].

In 2017 ASF was for the first time reported in Irkutsk, Omsk, Chelytabinsk, Kaliningrad, Tyumen, and Kurgan Oblast and according to the Rosselkhoznadzor in Yamalo-Nenets Autonomous District and Krasnoyarsk Krai [11]. Considering the territorial remoteness of the Subjects included in the Siberian and Urals Federal Districts from previously ASF infected RF Subjects we can suggest that the ASF agent as introduced to their territory with regulated commodities [10].

The majority of the ASFV genome-positive samples were from were from Tyumen Oblast (100.00%), Nizhny Novgorod Oblast (100.00%) and Omsk Oblast (91.51%) (Table 1).

Out of 8,500 samples sent for PCR-test 110 were samples of pathological material from wild boars (1.18%), which came from 6 Subjects of the Central, Southern, and North-Western Federal Districts of the Russian Federation with high density of the animal population (Table 2). According to the accompanying documents the samples were collected from shot animals and wild boar carcasses found in the forests.

The laboratory tests revealed ASFV genome in 17 (15.50%) out of 110 samples.

According to the previously published data the number of outbreaks in the wild fauna caused by ASF is 40% of all outbreaks [8]. To recognize the country as free from ASF it is necessary to provide evidence of the zero virus circulation in the population of susceptible animals (including wild boars) based on monitoring tests [7].

In this context further surveillance of ASF susceptible animal populations (domestic pigs and wild boars) is important.

Table 2
Results of RT-PCR-tests of samples collected from wild boars

No.	RF Subject	Total number of samples	Number of samples containing ASFV genome
1	***Belgorod Oblast***	2	**2 (100.00%)**
2	Bryansk Oblast	48	0
3	***Vladimir Oblast***	13	**1 (7.69%)**
4	***Volgograd Oblast***	1	**1 (100.00%)**
5	***Kaliningrad Oblast***	16	**12 (75.00%)**
6	***Republic of Crimea***	30	**1 (3.33%)**
	Total	**110**	17

CONCLUSION

The results of the tests performed as well as continuous ASF infection of the RF territory point to the necessity to continue monitoring using direct diagnosis methods (PCR). As according to the Governmental Programme most samples of the biological material are delivered only by the end of the year it can result in distortion of the actual disease spread situation and detection of timely measures for the infection outbreak containment. In this context the Subject should generate and timely implement the sampling schedule with equal time distribution of biomaterial sampling and sample transfer to the testing laboratories.

The test results revealed 188 ASF outbreaks both in new and previously free Subjects of the Siberian and Urals Federal Districts as well as in Subjects continuously infected with ASF (Krasnodar Krai, Rostov and Volgograd Oblasts). The obtained data correlate with the ASF spread forecast previously prepared by the specialists of the Information and Analysis Centre of the RF Veterinary Surveillance Service [8].

Wild boars are an additional ASF risk-group as the agent escape to non-protected domestic pig populations remains. Consequently it is necessary to develop and implement a programme for the disease surveillance in wild fauna. The presence of infected boars predetermines the necessity of systemic monitoring covering the habitat of these animals. Close attention shall be given to the habitats where human activities are observed including habitats located close to settlements, being a favorite site for hunting, gathering and recreation.

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